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Yes! Sex Matters: Sex, The Brain and Blood Pressure

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Abstract

The role of the brain in sex differences in hypertension is known to be important especially with regards to the effects of circulating sex hormones. A number of different brain regions important for regulation of sympathetic outflow and blood pressure express estrogen receptors (ER), ER α and ER β . Estradiol, acting predominantly via the ER α , inhibits angiotensin II activation of area postrema and subfornical organ neurons and inhibits reactive oxygen generation which is required for the development of Angiotensin II induced neurogenic hypertension. Estradiol activation of ER β within the paraventricular nucleus and the rostral ventral lateral medulla inhibits these neurons and inhibits angiotensin II or aldosterone induced increases in sympathetic outflow and hypertension. Understanding the cellular and molecular mechanisms underlying ER α and ER β actions within key brain regions regulating blood pressure will be essential for the development of “next generation” SERMS that can be used clinically for the treatment of neurogenic hypertension.

Keywords

Sex; gender; estradiol; estrogen receptors; hypertension; nucleus of the solitary tract; area postrema; subfornical organ; paraventricular nucleus; rostral ventral lateral medulla; reactive oxygen species; nitric oxide

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Compliance with Ethics Guidelines

Conflict of Interest

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INTRODUCTION

Over the last decade, considerable evidence has shown that sex does indeed matter with regards to the study of cardiovascular physiology and related diseases. There are significant sex differences in the development of hypertension and estradiol has been shown to modulate cardiovascular function at a number of different levels including the heart, vasculature, kidneys and brain [1], [2], [3], [4]. In humans, premenopausal women have been shown to have lower blood pressure as compared to age matched men [5]. However, after age 50, women have a significantly higher prevalence of hypertension as compared to men [6]. The role of gonadal hormones in the development of hypertension in older women is still controversial [4]. Some studies in postmenopausal women have shown that transdermal hormone replacement therapy (HRT) reduced mean blood pressure in normotensive women [7]. Yet, other studies of postmenopausal women on HRT for 5 years found no effect on resting blood pressure [8].

In a number of experimental models of hypertension, this sex dependent difference in the development of high blood pressure has also been observed [9], [10], [11], [12]. In the young spontaneously hypertensive rat (SHR) model, the sex difference in blood pressure is independent of estrogen status [13]. However, in the older female SHR animals that have ceased estrous cycling, the increase in blood pressure is similar to that seen in males [4]. Thus, the effects of both age and hormone status are likely to impact the development of hypertension.

In other models of hypertension, both sex and gonadal hormone status are important for the development of high blood pressure. In the Dahl-salt sensitive rat model of hypertension, intact males show an increase in blood pressure when exposed to high salt and intact females do not [10]. Castration prevents the increase in blood pressure in males [14] and ovariectomy exacerbates the increase in blood pressure in females [15] suggesting that both testosterone and estradiol are important modulators of hypertension. Estradiol replacement is effective at attenuating high blood pressure in both intact males and ovariectomized (OVX) females [10], [16], suggesting that estradiol has a protective role in both females and males against the development of hypertension [17], [19].

Although considerable evidence suggests that gonadal steroids play an important role in the development of hypertension, the role of sex chromosome effects (SCEs) and the interaction between gonadal hormones and sex chromosomes have been less thoroughly studied. Using the 4 core genotype (FCG) mouse model, it has been shown that there is a clear SCE on the development of hypertension in the Ang II model of high blood pressure [18]. In these studies with Ang II infusion, the MAP was greater in XX compared with XY mice regardless of whether the mice were born with testes or with ovaries. These authors, for the first time, were able to isolate the sex chromosome component from gonadal sex hormones and offer clear evidence for a sex chromosome difference in hypertension. Future studies are needed to identify the role of the SCE in brain sites involved in blood pressure regulation.

The underlying mechanisms of the sex differences in the development of hypertension and estradiol's suggested protective effects involve multiple end organs including the peripheral

vasculature, renal function and brain regions important for central regulation of sympathetic outflow [19],[9], [20]. The present review will focus on some of estradiol's action on brain regions known to be involved in the regulation of sympathetic outflow and blood pressure and some of the central cellular mechanisms thought to be involved in the differences between the sexes in the development of hypertension.

Role of the Brain in Sex Differences in Hypertension: Sympathetic Outflow and Baroreflex Function

The role of the central nervous system in estradiol's protection from Ang II and aldosterone dependent hypertension have been shown in studies where estradiol replacement was limited to the brain. Central intracerebroventricular (ICV) infusion of estradiol in ovariectomized (OVX) females and in male mice attenuates the pressor effect of Ang II and this effect is blocked if brain estrogen receptors are blocked [16]. Similarly, in aldosterone/salt-induced hypertension, sex differences observed in this model are normalized by blocking brain estrogen receptors in females or by activation brain estrogen receptors in males [21]. Thus, brain estrogen receptors in both males and females are able to modulate the development of multiple forms of hypertension.

Sympathetic Nervous System

The role of the sympathetic nervous system in sustaining increases in blood pressure is often indirectly determined by measuring the drop in blood pressure that occurs during autonomic ganglion blockade following administration of a ganglionic blocking agent such as hexamethonium. During infusion of Ang II in male and female mice, ganglionic blockade on day 7 of Ang II infusion produces a significantly smaller decrease in blood pressure in females compared with males, suggesting less activation of sympathetic nervous system by Ang II in females [11]. This Ang II induced increase in sympathetic outflow in males is consistent with previous reports via direct recordings of sympathetic outflow in male rats [22], [23]. To determine if circulating estradiol is involved in the sex differences in Ang II induced increases in sympathetic outflow the effects of ganglionic blockade after 7 days infusion of Ang II was tested in OVX vs intact female mice. The OVX females exhibit a greater ganglionic blockade induced drop in blood pressure as compared to intact female mice suggesting circulating levels of female gonadal hormones inhibits sympathetic outflow [24]. The role of estradiol actions on the brain to regulate sympathetic outflow have been demonstrated as well. In intact females receiving ICV administration of ER blocker ICI-182,780, ganglionic blockade at day 7 of Ang II infusion results in an increased drop in blood pressure compared to intact female mice with no ICV ER blocker or compared with OVX mice receiving ICV estradiol infusion [24]. Similar sex differences and estradiol brain actions on sympathetic outflow have also been shown in aldosterone/salt induced hypertension [21]. Ganglionic blockade after aldosterone induced hypertension results in a smaller reduction in blood pressure in intact females and in intact males treated with ICV estradiol as compared to intact males, castrated males, OVX females and intact females treated with ICV estradiol. These studies suggest that estradiol acts in the brain to inhibit sympathetic outflow in both male and female experimental models of hypertension.

Recent studies in the SHR rat have shown that older female SHR's have an increase in sympathetic outflow as compared to younger animals [25]. In these studies, the fall in blood pressure following renal denervation was greater in the older females as compared to their younger female counterparts suggesting that hypertension in older females in the SHR model of hypertension involves elevated sympathetic outflow.

The role of increases in sympathetic outflow in hypertension in aging women is still not clear. It has been shown that young women, compared to men, have lower resting sympathetic nerve activity [26]. However, how the levels of sympathetic nerve activity relate to total peripheral resistance differs between young men and young women. In young men there is a positive relationship between total peripheral resistance (TPR) and sympathetic outflow, but this relationship is absent in young women [26] suggesting that there are sex differences between young men and women in the mechanisms affecting TPR and blood pressure. This difference is lost as women enter menopause. Studies examining sympathetic activity in older men and women revealed that there is an increase in sympathetic activity in both sexes as they age and this increase is tightly coupled to increases in blood pressure [27]. Thus, the postmenopausal increase in sympathetic outflow in women most likely contributes to the increased incidence of hypertension in women over 50 years of age.

Baroreceptor Reflex

Sex differences in baroreceptor reflex function have been reported in humans [28], [29], [27]. Young women have been shown to have lower resting sympathetic outflow and enhanced baroreflex function as compared to age matched men [30]. In other studies using a variable pressure neck chamber technique to derive full carotid baroreflex stimulus-response curves, young women in the early follicular phase of the menstrual cycle, exhibit a greater bradycardic response and a greater depressor response to simulated carotid hypertension compared to young men [28]. The specific role of sex steroids and the sites of actions for these sex differences in baroreceptor function between men and women have not yet been determined.

The regulation of blood pressure by the baroreceptor reflex has also been shown to be different in male and female animal models. In male mice, the slope of ANG II-induced baroreflex bradycardia is significantly less than that induced by increasing blood pressure with phenylephrine. But, this Ang II-mediated attenuation of reflex bradycardia is not observed in the female mice [31]. It has been suggested that estradiol facilitates baroreflex function in female mice and that Ang II-mediated acute blunting of baroreflex regulation of heart rate may be sex dependent.

In animal studies, recent results have shown that sex chromosome complement has a significant influence on the cardiac baroreflex response to increases in blood pressure by phenylephrine and Ang II [32]. In these studies, the four core genotype mouse model was used in order to dissociate the effect of gonadal sex from sex hormones allowing comparisons of sexually dimorphic traits among XX and XY females, as well as in XX and XY males. The administration of phenylephrine in gonadectomized XY females resulted in a significantly lower baroreflex response when compared with the other genotypes. In

addition, in both gonadectomized XY males and females, the angiotensin II-bradycardic baroreflex response was attenuated when compared with heart rate changes in gonadectomized XX male and female mice. These data support the hypothesis that there are sex chromosome dependent differences in baroreflex function that are independent of gonadal hormones.

The molecular and cellular mechanisms within the brain underlying sex differences in Ang II modulation of the arterial baroreflex have not yet been fully defined. There are substantial data to suggest that sex steroids are involved in the sex differences observed with Ang II modulation of both blood pressure and baroreflex function. Estradiol replacement in OVX rats has been shown to decrease AT₁ receptor expression and binding affinity at several central sites including the SFO, a circumventricular organ and other areas that lie outside the blood-brain barrier [33],[34], [35], [36]. It has been hypothesized that AT₁ expression at central nuclei mediating the effects of Ang II on baroreflex function could be greater in males compared with estradiol replaced OVX females. This sex difference in AT₁ receptor expression might underlie the sex differences observed in Ang II modulation of the baroreceptor reflex. Additional biochemical and molecular studies are needed to test this hypothesis.

Brain Pathways Regulating Blood Pressure: Sites of Estrogen Action

Central brain regions involved in the regulation of sympathetic and parasympathetic neuronal activity include numerous brain stem and forebrain nuclei including the nucleus of the solitary tract (NTS), area postrema (AP), dorsal motor nucleus (DMV), nucleus ambiguus (NA), caudal ventral lateral medulla (CVLM), rostral ventral lateral medulla (RVLM), paraventricular nucleus of the hypothalamus (PVN), and the SFO. Importantly, each of these key structures involved in blood pressure regulation has been shown to express estrogen receptors [37], [38].

Area Postrema

The area postrema (AP), a circumventricular organ in the hindbrain, is well known to be involved in the neurohumoral regulation of sympathetic outflow and is modulated by circulating peptides [39], [40], [41]. Lesioning of the AP facilitates Ang II-induced baroreflex control of heart rate and normalizes the slope of the Ang II-induced arterial pressure-heart rate curve to that seen with phenylephrine, suggesting that the AP is involved in the central actions of Ang II on baroreflex control of heart rate [40]. Angiotensin II also increases [Ca⁺⁺]_i signaling in neurons and this increase has been shown to require reactive oxygen signaling [42]. At the level of the AP, Ang II increases the [Ca⁺⁺]_i in neurons and this response is inhibited by pre-incubation of neurons with estradiol [43]. In vivo electrophysiological studies revealed that AP neuronal activity is inhibited by estradiol application [44]. Further, in patch-clamp studies of isolated AP neurons, estradiol increased a calcium-activated K⁺ channel [44] which contributes to estradiol's inhibition of AP neuronal activity.

Nucleus of the Solitary Tract

In the brainstem, the NTS is known to receive afferent input from peripheral baroreceptor and chemoreceptor neurons and is essential for the integration of these sensory inputs and the function of the baroreceptor reflex. The NTS expresses both ER α and ER β receptors [45], [46], however, ER α is the more predominant form. Expression of the ER α receptor is correlated to levels of circulating estrogen during the estrous cycle [46]. In these studies using real-time PCR, ER α mRNA expression in the NTS was highest during estrus, and lowest levels in metestrus.

The direct effects of estradiol actions at the level of the NTS have been investigated using anesthetized preparations. In male rats, microinjection of estradiol into the NTS increases efferent vagal activity and decreases sympathetic tone [47]. Interestingly, in experiments utilizing iontophoretic application and extracellular recordings of NTS neuronal activity, estradiol inhibits both spontaneous and glutamate receptor evoked NTS neuronal activity [48]. The inhibitory effects of estradiol in these studies were observed within one minute and were rapidly reversed suggesting the possible involvement of a non-genomic estrogen receptor at the level of the NTS. The inhibitory action of estradiol on NTS neurons is contrary to what might be expected if estradiol injections into the NTS inhibit sympathetic outflow and blood pressure. One might anticipate that an inhibitory effect of estradiol at the level of the NTS would be associated with an increase in sympathetic activity. However, the chronic effects of estradiol on NTS neuronal activity have not been investigated. Whether the effects of estradiol in the NTS to regulate blood pressure involve classic genomic estrogen receptors, non-genomic membrane receptors such as GRP-30 [49] or both is unknown.

Interestingly estrogen receptors within the NTS, are regulated by Ang II induced hypertension [50]. In intact female rats, Ang II induced hypertension increases ER α receptor expression within the NTS but not ER β suggesting that Ang II hypertension alters ER α receptor expression and subcellular distribution which may contribute to known sex differences in Ang II induced hypertension.

Rostral Ventral Lateral Medulla

The rostral ventral lateral medulla (RVLM) expresses both ER α and ER β receptors, however, the ER β receptors and not ER α receptors with the RVLM contribute to the hypertensive protective effects of estradiol during aldosterone-induced hypertension [51]. In studies using siRNA to selectively knockdown either ER α or ER β within the RVLM of intact female rats infused with low doses of aldosterone, knockdown of ER β receptors within the RVLM resulted in a hypertensive effect of aldosterone in these intact females. Thus, it appears that ER β within the RVLM is a site of action for estradiol antihypertensive effects in the brain.

In whole-cell patch clamp recordings from isolated bulbospinal RVLM neurons, estradiol directly reduces voltage-gated calcium currents [52]. In these studies, estradiol dose-dependently reduced voltage-gated calcium currents, especially the long-lasting (L-type) component. This inhibition was reversed by washing or prevented by adding the non-

subtype-selective ER antagonist ICI182780. An ER β -selective agonist, but not an ER α -selective agonist, reproduced the calcium current inhibition. These data indicate that estrogens can modulate the function of RVLM neurons directly through activation of ER β . This calcium current inhibition may underlie the decrease in sympathetic tone evoked by local estradiol application and may be the site of action for estradiol's antihypertensive effects in the brain.

A potential cellular pathway that may be involved in ER inhibition of RVLM activity and vasodepressor effects has been suggested to involve the PI3K/Akt signaling pathway [53]. Inhibition of both PI3K and Akt signaling via microinjection of selective PI3K and AKT inhibitor into the RVLM was found to inhibit the vasodepressor effects of selective ER β activation.

Subfornical Organ

The SFO is a forebrain circumventricular organ and its primary projections to the PVN are thought to be a key pathway involved in Ang II mediated increases in sympathetic outflow and hypertension. Increases in circulating Ang II increases the firing rate of SFO neurons [54],[55] and the SFO also expresses estrogen receptors which have been shown to be co-localized on the same neurons that express AT1 receptors [56]. Recent electrophysiological studies in anesthetized rats have shown that chronic administration of estradiol inhibits both the basal firing rate of SFO neurons and inhibits the Ang II excitatory effects on these same neurons [35]. These studies suggest that the sex differences seen with Ang II hypertension involves estradiol's inhibition of the actions of circulating Ang II on central neurons which are required for the development of this form of neurogenic hypertension.

A critical cellular mechanism underlying the effects of Ang II at the level of the SFO involves the generation of ROS [42]. Zimmerman and colleagues [57], [42] have shown that Ang II induced hypertension requires activation of NADPH oxidase and the production of ROS. In SFO brain slices [16] and in isolated neurons [57], application of ANG II has been shown to increase ROS production. Treatment of SFO slices with estradiol inhibits Ang II induced increases in ROS suggesting that the central actions of estradiol to protect from ANG II-induced hypertension may involve estradiol inhibition of ROS production [16].

Paraventricular Nucleus

The PVN is an important region for the integration of brain pathways involved in whole body fluid regulation and sympathetic outflow. Anatomically, the PVN contains magnocellular and parvocellular neurons that are divided into distinct regions with specific and functionally unique projections. A subset of parvocellular neurons of the PVN contribute to the regulation of sympathetic outflow. It receives key information about circulating peptide levels from the SFO as well as input from the NTS and caudal ventral lateral medulla (CVLM). In turn, PVN neurons project to the RVLM and the intermediolateral column of the spinal cord region which controls sympathetic nerve activity.

The ER β is the principal ER expressed in PVN neurons [58], [59]. Recent studies using siRNA-knockdown of ER β within the PVN show that repressing the expression of ER β increases aldosterone induced hypertension in intact females [51]. These results suggest that

the ER β in the PVN is partially responsible for protecting females from the central effects of aldosterone to increase sympathetic outflow and blood pressure.

The cellular mechanism underlying these effects of estradiol on PVN neurons has been suggested to involve estradiol inhibition of ROS production. Indeed, a knock-down of ER β resulted in an increase in aldosterone induced ROS production in PVN neurons suggesting that ER β may modulate elements within the NADPH-oxidase pathway [51].

Nitric oxide (NO) is also thought to be an important intracellular regulator of PVN activity [60]. Increases in sympathetic outflow during hypertension and congestive heart failure have been shown to involve decreases in PVN NO production [61], [60]. Estradiol has been found to modulate nNOS within the PVN [62]. In these studies using anesthetized male rats, the pressor effects caused by microinjection of l-glutamate in the PVN are inhibited by pretreatment with estradiol. This capacity of estradiol to prevent increases in blood pressure caused by glutamate receptor activation is blocked by ER β receptor inhibition but not by ER α inhibition further supporting a role for ER β in the PVN. Further, the blood pressure protective effects of estradiol within the PVN are also blocked if the PVN is pretreated with a NOS inhibitor suggesting that estradiol may inhibit activation of PVN neurons by increasing NOS and NO production [62].

Similarly, central blockade of nNOS has also been shown to increase Ang II induced increases in sympathetic outflow and hypertension in intact females but not males [63]. Further, expression of nNOS within the PVN and the SFO is higher in intact females compared to males and ovariectomy eliminates the increase in nNOS expression observed in females [63]. These results further support the interpretation that a key cellular mechanism involved in the central actions of estradiol's action to protect high blood pressure most likely involves estradiol modulation of brain NO production.

Recent studies using an elegant transgenic mouse model expressing enhanced green fluorescent protein (EGFP) in ER β -containing cells examined the roles of both sex and age on NMDA NR1 subunit trafficking in ER β positive dendrites in the PVN [64]. This group reported that during Ang II induced hypertension, young females have a decrease in NR1 density as compared to males or older females suggesting that young female mice may be protected from Ang II hypertension because of reduced NMDA receptor activity within the PVN and thus reduced increases in sympathetic outflow and hypertension.

Conclusion

Figure 1A illustrates some of the important brain sites of action for estradiol modulation of sympathetic activation and ultimately neurogenic hypertension. Key sites include two circumventricular organs, the AP and the SFO, which are known to be sites of action for circulating peptides, such as Ang II, to induce increases in sympathetic outflow and hypertension. Estradiol inhibits firing of neurons in these circumventricular neurons and inhibits their activation by circulating peptides. At the level of the NTS, estradiol can directly modulate neuronal activity, baroreceptor afferent information integration and ultimately arterial baroreflex function. Estradiol also has important effects on sympathetic

pre-motor neuron drivers in the PVN and the RVLM and is able to inhibit the activity of these neurons involved in driving sympathetic outflow. The inhibition of the neurons may be how estradiol protects from some forms of neurogenic hypertension. Layered on to the complexity of these multiple central sites for estradiol action is the discrete expression of ER α or ER β receptors. Both receptors are known to bind to estrogen response elements (ERE) and act to modulate gene function and protein expression of specific proteins such as the AT1 receptor and NOS. In addition, non-genomic actions of ER activation have also been shown in some neurons and may be responsible for the fast actions of estradiol to inhibit AP and NTS neuronal activity. While most of the cardiovascular regulatory regions of the brain seem to express both receptors, the ER β has been shown to be key in estradiol actions on many structures lying inside the blood-brain barrier to affect efferent sympathetic tone while ER α has a more prominent role at the circumventricular organs and the NTS.

Figure 1B summarizes the effects of sex and brain applied estradiol in the development of hypertension (either Ang II or aldosterone-induced) in male and female animal models. Intact females are protected from the development of hypertension relative to intact males. Removal of ovaries results in hypertension in females. Activation of central estrogen receptors inhibits hypertension development in OVX females and in males. Blockade of ER β within the PVN or RVLM increases hypertension development in intact females and normalizes the sex differences between intact males and intact females. Lastly, inhibition of brain NOS in intact females results in hypertension while decreases in brain ROS formation inhibits hypertension in males.

There is a tremendous amount that is still unknown about the central cellular mechanisms underlying sex differences in hypertension. For example, while evidence is clear that the sex chromosome complement is very important in the sex differences in some forms of hypertension, how these genes modulate the neurons involved in controlling sympathetic outflow and blood pressure and how these effects are altered (or not) by gonadal hormones is still unclear.

Lastly, translating these studies in preclinical animal models to affecting clinical antihypertensive therapies in humans, both men and women, should continue to be the ultimate goal. For example, it is currently unknown if systemic treatment with “next generation” receptor selective ER antagonists (SERMS) can be targeted to act on discrete brain regions. If new therapies could be designed to only act on ERs within circumventricular organs, then some of side effects of systemic activation of ER α or ER β receptors might be avoided. Realization of truly transformative, individualized therapies for the treatment of neurogenic hypertension will require a comprehensive integration of the cellular mechanisms underlying estradiol’s actions on brain regions and other peripheral organs critical for blood pressure regulation.

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Effects of Estradiol (E2) on Brain Regions Regulating Blood Pressure

Figure 1.

Stylized representation of the hypothesis for how estradiol actions in the brain modulate the development of hypertension. (A) Estradiol acts at multiple sites within the brain to modulate sympathetic activity and blood pressure. Estradiol 1) inhibits neuronal activity at the AP, SFO and the NTS possibly via non-genomic ER receptor actions, 2) inhibits angiotensin II and aldosterone induced increases in iCa^{++} in both the SFO and the AP most likely acting via ER α actions on estrogen response elements (ERE) of estrogen modulated genes, 3) inhibits Ang II induced ROS formation in the SFO and the PVN, 4) increases

nNOS expression in the SFO and PVN, and 5) activates ER β within the PVN and RVLM to inhibit the development of hypertension. **(B)** Stylized summary of the sex differences between intact females and males in the development of experimental hypertension induced by low-dose infusion of either Ang II or aldosterone/salt. Illustrated are the effects of ovariectomy (OVX), and the effects brain intracerebroventricular (ICV) infusion of 1) estradiol in males and OVX females, 2) ER β inhibition in the PVN and RVLM of intact females, 3) NOS inhibition in intact females, and 4) ROS inhibition in intact males on the development of experimental hypertension. The vertical dotted line at time zero indicates the start of infusion of low dose of Ang II or aldosterone/salt. AP—area postrema; CVLM—caudal ventral lateral medulla; ERE-estrogen response element; E2-estradiol; ICV—intracerebroventricle; IML—interomediolateral cell column; nNOS-neuronal nitric oxide synthase; NTS—nucleus tractus solitarius; PVN—paraventricular nucleus; ROS-reactive oxygen species; RVLM—rostral ventrolateral medulla; SFO—subfornical organ.